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=> s CD39 fusion

L1 0 CD39 FUSION

=> s CD39

L2 1228 CD39

=> s 12 and soluble

L3 163 L2 AND SOLUBLE

=> s 13 and IL-2 leader

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=> dup remove 16

PROCESSING COMPLETED FOR L6

L7 6 DUP REMOVE L6 (0 DUPLICATES REMOVED)

=> d 17 1-6 cbib abs

L7 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
2002:676043 Document No. 137:213257 Increased recovery of active proteins using a reduction/oxidation coupling reagent. Sassenfeld, Helmut M.; Remmele, Richard L., Jr.; McCoy, Rebecca E. (Immunex Corporation, USA). PCT Int. Appl. WO 2002068455 A2 20020906, 38 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US5645 20020222. PRIORITY: US 2001-PV271033 20010223.

AB The invention provides methods of increasing yields of desired conformation of proteins. In particular embodiments, the invention includes contacting prepns. of a recombinant protein with a reduction/oxidation coupling reagent for a time sufficient to increase the relative proportion

of a desired configurational isomer. A TNF receptor-Fc fusion protein fraction with low TNF binding activity was treated with a redox coupling system of reduced glutathione and glutathione to drive the inactive form to the active conformation.

- L7 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN 2002:11124 Document No. 136:79766 Inhibitors of platelet activation and recruitment. Maliszewski, Charles Richard; Gayle, Richard Brownley; Price, Virginia Lee; Gimpel, Steven Dean (USA). U.S. Pat. Appl. Publ. US 2002002277 A1 20020103, 78 pp., Cont.-in-part of Appl. No. PCT/US99/22955. (English). CODEN: USXXCO. APPLICATION: US 2001-835147 20010413. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813; WO 1999-US22955 19991013.
- AB The present invention provides soluble CD39 polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble CD39 polypeptide.
- L7 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN

 2002:6330 Document No. 136:84678 Methods and materials relating to

 CD39-like polypeptides. Ford, John; Mulero, Julio J.; Yeung,

 George (Hyseq, Inc., USA). U.S. US 6335013 B1 20020101, 98 pp.,

 Cont.-in-part of U. S. Ser. No 583,231. (English). CODEN: USXXAM.

 APPLICATION: US 2000-608285 20000630. PRIORITY: US 1999-273447 19990319;

 US 1999-350836 19990709; WO 1999-US16180 19990716; US 1999-370265

 19990809; US 2000-481238 20000111; US 2000-557800 20000425; US 2000-583231

 20000526.
- The invention provides novel polynucleotides isolated from cDNA libraries of human fetal liver-spleen and macrophage as well as polypeptides encoded by these polynucleotides and mutants or variants thereof. The polypeptides correspond to a novel human CD39-like protein. Other aspects of the invention include vectors containing polynucleotides of the invention and related host cells as well a processes for producing novel CD39-like polypeptides, and antibodies specific for such polypeptides.
- L7 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN

 2000:277996 Document No. 132:303497 CD39 polypeptides as inhibitors of platelet activation and recruitment. Maliszewski, Charles R.; Gayle, Richard B., III; Price, Virginia L.; Gimpel, Steven D. (Immunex Corp., USA). PCT Int. Appl. WO 2000023459 A1 20000427, 122 pp.

 DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US22955 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.
- AB The present invention provides soluble CD39 polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble CD39 polypeptide.
- L7 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
 2000:277866 Document No. 132:303495 Methods of inhibiting platelet
 activation and recruitment. Maliszewski, Charles R.; Gayle, Richard B.,
 III; Marcus, Aaron J. (Immunex Corp., USA; Cornell Research Foundation,
 Inc.). PCT Int. Appl. WO 2000023094 A2 20000427, 118 pp. DESIGNATED
 STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR,
 CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN,
 IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,
 MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,
 TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
 TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
 GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English).
 CODEN: PIXXD2. APPLICATION: WO 1999-US23641 19991013. PRIORITY: US

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1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010
    19990813.
    The present invention provides soluble CD39 polypeptides
    and compns., and methods for inhibiting platelet activation and
    recruitment in a mammal comprising administering a soluble
     CD39 polypeptide.
    ANSWER 6 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN
            Document No. 132:135506 Identification of novel homologs of
     CD39 antigens of human and cDNAs encoding them. Ford, John;
    Mulero, Julio (Hyseq, Inc., USA). PCT Int. Appl. WO 2000004041 A2
     20000127, 125 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB,
    BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM,
     HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
    LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
    SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ,
     MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES,
    FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.
     (English). CODEN: PIXXD2. APPLICATION: WO 1999-US16180 19990716.
     PRIORITY: US 1998-118205 19980716; US 1998-122449 19980724; US 1999-244444
     19990204; US 1999-273447 19990319; US 1999-350836 19990709.
     CDNAs for homologs of the human CD39 antigen are cloned from
     cDNA libraries of human fetal liver-spleen and macrophage and the gene
     products are characterized. The proteins may be of use in the treatment
     of clotting disorders including thrombosis. Preliminary clones were
     obtained from a human fetal liver spleen cDNA library by determination of a
     sequence signature sequence followed by sequencing of those clones with
     CD39-like signatures. The clone obtained encoded a CD39
     -like protein and hybridized to an mRNA from macrophages, but not from any
     other tissue tested. Unlike CD39, this protein was sol
     . and secreted from cells and was shown to be an apyrase. It also shared
     conserved sequences with other apyrases and mutation of the conserved
     regions affected the apyrase activity.
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L10 ANSWER 1 OF 35 CAPLUS COPYRIGHT 2005 ACS on STN
           Document No. 136:79766 Inhibitors of platelet activation and
     recruitment. Maliszewski, Charles Richard; Gayle, Richard
     Brownley; Price, Virginia Lee; Gimpel, Steven Dean
     (USA). U.S. Pat. Appl. Publ. US 2002002277 A1 20020103, 78 pp.,
     Cont.-in-part of Appl. No. PCT/US99/22955. (English). CODEN: USXXCO.
     APPLICATION: US 2001-835147 20010413. PRIORITY: US 1998-PV104585
     19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813; WO
     1999-US22955 19991013.
     The present invention provides soluble CD39 polypeptides and
     compns., and methods for inhibiting platelet activation and recruitment in
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a mammal comprising administering a soluble CD39 polypeptide.

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- 2002230961. PubMed ID: 11956240. Elucidation of the thromboregulatory role of CD39/ectoapyrase in the ischemic brain. Pinsky David J; Broekman M Johan; Peschon Jacques J; Stocking Kim L; Fujita Tomoyuki; Ramasamy Ravichandran; Connolly E Sander Jr; Huang Judy; Kiss Szilard; Zhang Yuan; Choudhri Tanvir F; McTaggart Ryan A; Liao Hui; Drosopoulos Joan H F; Price Virginia L; Marcus Aaron J; Maliszewski Charles R. (Division of Cardiology, Department of Medicine, College of Physicians and Surgeons, Columbia University, Presbyterial Hospital 10 Stem, 630 W 168th Street, New York, NY 10032, USA.. djp5@columbia.edu) Journal of clinical investigation, (2002 Apr) 109 (8) 1031-40. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
- Endothelial CD39 metabolizes ADP released from activated AB platelets. Recombinant soluble human CD39 (solCD39) potently inhibited ex vivo platelet aggregation in response to ADP and reduced cerebral infarct volumes in mice following transient middle cerebral artery occlusion, even when given 3 hours after stroke. Postischemic platelet and fibrin deposition were decreased and perfusion increased without increasing intracerebral hemorrhage. In contrast, aspirin did not increase postischemic blood flow or reduce infarction volume, but did increase intracerebral hemorrhage. Mice lacking the enzymatically active extracellular portion of the CD39 molecule were generated by replacement of exons 4-6 (apyrase-conserved regions 2-4) with a PGKneo cassette. Although CD39 mRNA 3' of the neomycin cassette insertion site was detected, brains from these mice lacked both apyrase activity and CD39 immunoreactivity.. Although their baseline phenotype, hematological profiles, and bleeding times were normal, cd39(-/-) mice exhibited increased cerebral infarct volumes and reduced postischemic perfusion. solCD39 reconstituted these mice, restoring postischemic cerebral perfusion and rescuing them from cerebral injury. These data demonstrate that CD39 exerts a protective thromboregulatory function in stroke.
- L10 ANSWER 3 OF 35 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
- 2002017949 EMBASE Inhibition of platelet recruitment by endothelial cell CD39/ecto-ADPase: Significance for occlusive vascular diseases.

 Marcus A.J.; Broekman M.J.; Drosopoulos J.H.F.; Pinsky D.J.; Islam N.;

 Maliszewski C.R.. Dr. A.J. Marcus, Hematology/Medical Oncology, VA

 New York Harbor Healthcare System, Weill Med. College of Cornell Univ.,
 423 East 23rd Street, New York, NY 10010, United States.

 ajmarcus@med.cornell.edu. Italian Heart Journal 2/11 (824-830) 2001.

 Refs: 41.

ISSN: 1129-471X. CODEN: IHJOAM. Pub. Country: Italy. Language: English. Summary Language: English.

During their 7-9 day lifespan in the circulation platelets are mainly AB responsible for maintaining the integrity of the vasculature. In thrombocytopenic states, there is an increase in vascular permeability and fragility, presumably due to absence of this platelet function. In sharp contrast, biochemical or physical injury in the coronary, carotid or peripheral arteries induces platelet activation and platelet recruitment, which can culminate in thrombotic vascular occlusion. Since there is one death every 33 s from vascular occlusion in the United States, this situation constitutes a major public health issue. In the course of studying interactions between cells of the vascular wall and those in the circulation, we observed that platelets in close proximity to endothelial cells do not respond to agonists in vitro. Experiments initiated in the late 1980's cumulatively indicated that endothelial cell CD39 an ecto-ADPase - was mainly responsible for this phenomenon. CD39 rapidly and preferentially metabolizes ADP released from activated platelets. ADP is the final common pathway for platelet recruitment and thrombus formation, and platelet aggregation and recruitment are abolished by CD39. Our current hypothesis is that CD39 will be a novel antithrombotic agent for treating high risk patients who have activated platelets in their circulation - the identifying characteristic of coronary artery occlusion and thrombotic stroke. A recombinant, soluble form of human CD39 has been generated. This is solCD39, a glycosylated protein of 66 kDa whose enzymatic and biological properties are identical to the full-length form of the enzyme. In our in vitro

experiments, solCD39 blocks ADP-induced human platelet aggregation, and inhibits collagen- and thrombin receptor agonist peptide-induced platelet reactivity. We studied solCD39 in vitro in a murine model of stroke, which was shown to be driven by excessive platelet recruitment. In studies with CD39 wild-type (CD39(+/+)) mice solCD39 completely abolished ADP-induced platelet aggregation, and strongly inhibited collagen- and arachidonate-induced platelet reactivity ex vivo. When solCD39 was administered prior to transient intraluminal middle cerebral artery occlusion, it reduced ipsilateral fibrin deposition, decreased (111) In-platelet deposition, and increased post-ischemic blood flow 2-fold at 24 hours. These results were superior to those we obtained with aspirin pre-treatment. CD39 null (CD39(-/-)) mice, which we generated by deletion of exons 4-6 (apyrase conserved regions 2-4), have a normal phenotype, normal hematologic profiles and bleeding times, but exhibit a decrease in post-ischemic perfusion and an increase in cerebral infarct volume when compared to genotypic CD39(+/+) controls in our stroke model. "Reconstitution" of CD39 null mice with solCD39 reversed these pathologic changes. Thus, the CD39(-/-) mice were actually rescued from cerebral injury by solCD39, thereby fulfilling Koch's postulates. These experiments have led us to hypothesize that solCD39 has potential as a novel therapeutic agent for thrombotic stroke. In this review, we summarize our recent research results with CD39 and solCD39, and discuss our viewpoints on its present and future possibilities as a novel treatment for thrombosis.

- L10 ANSWER 4 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 2001:214774 The Genuine Article (R) Number: 389JF. Soluble CD39 but not aspirin decreases platelet deposition and improves outcome in reperfused murine stroke. Kiss S (Reprint); Marcus A J; Broekman M J; Nair M N; D'Ambrosio A L; Liao H; Maliszewski C R; Connolly E S; Pinsky D J. Columbia Univ Coll Phys & Surg, New York, NY 10032 USA; Cornell Univ, Weill Med Coll, New York, NY USA; Immunex Res & Dev Corp, Seattle, WA 98101 USA. STROKE (JAN 2001) Vol. 32, No. 1, pp. 359-359. MA P109. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 0039-2499. Pub. country: USA. Language: English.
- L10 ANSWER 5 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 2001:936710 The Genuine Article (R) Number: 487UW. Leukoregulatory role of CD39 in ischemic vessels. Fujita T (Reprint); Maliszewski C R; Liao H; Okada K; Marcus A J; Broekman M J; Ramasamy R; Pinsky D J. Columbia Univ, New York, NY USA; Immunex Corp, Seattle, WA USA; Columbia Univ, Weill Med Coll, New York, NY USA. CIRCULATION (23 OCT 2001) Vol. 104, No. 17, Supp. [S], pp. 274-274. MA 1316. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 0009-7322. Pub. country: USA. Language: English.
- L10 ANSWER 6 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 2
- 2001:251364 The Genuine Article (R) Number: 412PH. Thromboregulation by endothelial cells Significance for occlusive vascular diseases. Marcus A J (Reprint); Broekman M J; Drosopoulos J H F; Pinsky D J; Islam N; Gayle R B; Maliszewski C R. VA New York Harbor Healthcare Syst, 423 E 23rd St, Room 13028W, New York, NY 10010 USA (Reprint); VA New York Harbor Healthcare Syst, New York, NY 10010 USA; Cornell Univ, Weill Med Coll, New York, NY USA; Columbia Univ Coll Phys & Surg, New York, NY 10032 USA; Immunex Corp, Seattle, WA USA. ARTERIOSCLERO SIS THROMBOSIS AND VASCULAR BIOLOGY (FEB 2001) Vol. 21, No. 2, pp. 178-182. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 1079-5642. Pub. country: USA. Language: English.*

During their 7- to 9-day lifespan in the circulation, platelets perform an ill-defined baseline function that maintains the integrity of the vasculature. In thrombocytopenic states, there is an increase in vascular permeability and fragility, which is presumably due to absence of this platelet function. In sharp contrast, biochemical or physical injury in the coronary, carotid, or peripheral arteries induces platelet activation

and platelet recruitment, which can progress to thrombotic vascular occlusion. Because there is 1 death every 33 seconds from vascular occlusion in the United States, this problem has critical public health implications. In this review, we describe the characterization of a novel potential antithrombotic agent with a unique mode of action--biochemical "deletion" of ADP from an activated platelet releasate, which thereby inhibits platelet recruitment and further activation.

L10 ANSWER 7 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN 2002:274993 Document No.: PREV200200274993. Leukoregulatory role of CD39 in ischemic vessels. Fujita, Tomoyuki [Reprint author];

Maliszewski, Charles R.; Liao, Hui; Okada, Kenji; Marcus, Aaron J.; Broekman, M. Johann; Ramasamy, Ravichandran; Pinsky, David J.. Columbia Univ, New York, NY, USA. Circulation, (October 23, 2001) Vol. 104, No. 17 Supplement, pp. II.274. print.

Meeting Info.: Scientific Sessions 2001 of the American Heart Association. Anaheim, California, USA. November 11-14, 2001. American Heart Association.

CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.

L10 ANSWER 8 OF 35 CAPLUS COPYRIGHT 2005 ACS on STN 2000:277996 Document No. 132:303497 CD39 polypeptides as

inhibitors of platelet activation and recruitment. Maliszewski,

Charles R.; Gayle, Richard B., III; Price, Virginia

L.; Gimpel, Steven D. (Immunex Corp., USA). PCT Int. Appl.

WO 2000023459 Al 20000427, 122 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US22955 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106; US 1999-PV149010 19990813.

- The present invention provides soluble CD39 polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble CD39 polypeptide.
- L10 ANSWER 9 OF 35 CAPLUS COPYRIGHT 2005 ACS on STN

 2000:277866 Document No. 132:303495 Methods of inhibiting platelet
 activation and recruitment. Maliszewski, Charles R.;
 Gayle, Richard B., III; Marcus, Aaron J. (Immunex Corp., USA;
 Cornell Research Foundation, Inc.). PCT Int. Appl. WO 2000023094 A2
 20000427, 118 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB,
 BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE,
 GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
 LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE,
 SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM,
 AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM,
 CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT,
 SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US23641
 19991013. PRIORITY: US 1998-PV104585 19981016; US 1998-PV107466 19981106;
 US 1999-PV149010 19990813.
- The present invention provides soluble CD39 polypeptides and compns., and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble CD39 polypeptide.
- L10 ANSWER 10 OF 35 MEDLINE on STN DUPLICATE 3
 2000302517. PubMed ID: 10841775. Site-directed mutagenesis of human endothelial cell ecto-ADPase/soluble CD39: requirement of glutamate 174 and serine 218 for enzyme activity and inhibition of platelet recruitment. Drosopoulos J H; Broekman M J; Islam N;
 Maliszewski C R; Gayle R B 3rd; Marcus A J. (Department of Medicine, Division of Hematology and Medical Oncology, VA New York Harbor Healthcare System, New York, New York 10010-5050, USA.. jhfliess@mail.med.cornell.edu) . Biochemistry, (2000 Jun 13) 39 (23) 6936-43. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

- Endothelial cell CD39/ecto-ADPase plays a major role in vascular AΒ homeostasis. It rapidly metabolizes ADP released from stimulated platelets, thereby preventing further platelet activation and recruitment. We recently developed a recombinant, soluble form of human CD39, solCD39, with enzymatic and biological properties identical to To identify amino acids essential for enzymatic/biological activity, we performed site-directed mutagenesis within the four highly conserved apyrase regions of solCD39. Mutation of glutamate 174 to alanine (E174A) and serine 218 to alanine (S218A) resulted in complete and approximately 90% loss of solCD39 enzymatic activity, respectively. Furthermore, compared to wild-type, S57A exhibited a 2-fold increase in ADPase activity without change in ATPase activity, while the tyrosine 127 to alanine (Y127A) mutant lost 50-60% of both ADPase and ATPase activity. The ADPase activity of wild-type solCD39 and each mutant, except for R135A, was greater with calcium as the required divalent cation than with magnesium, but for ATPase activity generally no such preference was observed. Y127A demonstrated the highest calcium/magnesium ADPase activity ratio, 2.8-fold higher than that of wild-type, even though its enzyme activity was greatly reduced. SolCD39 mutants were further characterized by correlating enzymatic with biological activity in an in vitro platelet aggregation system. Each solCD39 mutant was similar to wild-type in reversing platelet aggregation, except for E174A and S218A. E174A, completely devoid of enzymatic activity, failed to inhibit platelet responsiveness, as anticipated. S218A, with 91% loss of ADPase activity, could still reverse platelet aggregation, albeit much less effectively than wild-type solCD39. Thus, glutamate 174 and serine 218 are essential for both the enzymatic and biological activity of solCD39.
- L10 ANSWER 11 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on DUPLICATE 4
- 2001:322309 Document No.: PREV200100322309. Identification of functionally
 important amino acid residues in soluble human CD39: An
 important thrombo-regulator. Drosopoulos, J. H. F. [Reprint author];
 Broekman, M. J.; Islam, N.; Gayle, R. B., III; Maliszewski,
 C. R.; Marcus, A. J.. VA NY Harbor Healthcare System, Weill Medical
 College of Cornell Univ., New York, NY, USA. Blood, (November 16, 2000)
 Vol. 96, No. 11 Part 1, pp. 813a. print.
 Meeting Info.: 42nd Annual Meeting of the American Society of Hematology.
 San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
- CODEN: BLOOAW. ISSN: 0006-4971. Language: English. Endothelial cell ecto-ADPase/CD39 plays a major role in AΒ maintenance of blood fluidity. It rapidly metabolizes ADP released from activated platelets, thereby preventing further platelet activation and recruitment. We developed a recombinant, soluble form of human CD39, solCD39, with enzymatic and biological properties identical to CD39. To identify amino acids essential for enzymatic/biological activity, we performed site-directed mutagenesis within the highly conserved apyrase regions (ACR) of solCD39. Mutations E174A and S218A resulted in complete and apprx90% loss of enzymatic activity, respectively. Compared to wild-type, S57A displayed a 2-fold increase in ADPase activity with no change in ATPase activity, whereas Y127A lost apprx55% of both ADPase and ATPase activity. D213A showed the greatest increase in both ADPase and ATPase activity. D54A and D213A had 1.5-fold higher enzyme activity with ATP than with ADP as substrate. Enzymatic activity of solCD39 mutants correlated strongly with their biological activity in an in vitro platelet aggregation system. citrated plasma, each mutant resembled wild-type in reversing platelet aggregation, with the exception of D54A, E174A, D213A, and S218A. devoid of enzyme activity, did not inhibit platelet reactivity. S218A, with 91% loss of ADPase activity, could still reverse platelet aggregation, albeit much less effectively than wild-type. Interestingly, D54A and D213A had decreased ability to reverse platelet aggregation, even through their ADPase and ATPase activities were greater than that of wild-type in enzymatic assays. In addition, their ADPase activity in the presence of citrated plasma was also decreased, and this was overcome by addition of excess calcium. The citrate in anticoagulated plasma reduced free calcium to a suboptimal level for full enzymatic activity of D54A and D213A, and decreased their ability to inhibit platelet aggregation as

effectively as wild-type solCD39. In heparinized plasma, D54A and D213A completely reversed platelet aggregation and their ADPase activities were similar to that observed in enzyme assays. Kinetic analyses revealed a low binding affinity of D54A and D213A for calcium as well as for ADP and ATP. Decreases in binding were compensated for by increases in rate of catalysis. Thus, aspartates 54 and 213 are involved in calcium binding in the catalytic pocket of solCD39. Glutamate 174 and serine 218 are essential for the enzymatic as well as biological activity of the enzyme. Our study defines amino acid residues required for enzyme catalysis and provides specific information concerning the active site of solCD39 - a potential antithrombotic agent.

- L10 ANSWER 12 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 2001:53830 The Genuine Article (R) Number: 367QE. Inhibition of platelet aggregation with CD39: An ex vivo dose response study. Buergler J M (Reprint); Kaluza G L; Maliszewski C R; Ali N M. Baylor Coll Med, Houston, TX 77030 USA; Immunex Res & Dev Corp, Seattle, WA 98101 USA. CIRCULATION (31 OCT 2000) Vol. 102, No. 18, Supp. [S], pp. 131-131. MA 630. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. ISSN: 0009-7322. Pub. country: USA. Language: English.
- L10 ANSWER 13 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 2001:110428 Document No.: PREV200100110428. Inhibition of platelet aggregation with CD39: An ex vivo dose response study. Buergler, John M. [Reprint author]; Kaluza, Grzegorz L. [Reprint author]; Maliszewski, Charles R.; Ali, Nadir M. Baylor Coll of Medicine, Houston, TX, USA. Circulation, (October 31, 2000) Vol. 102, No. 18 Supplement, pp. II.131. print.

Meeting Info.: Abstracts from American Heart Association Scientific Sessions 2000. New Orleans, Louisiana, USA. November 12-15, 2000. American Heart Association.

CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.

- L10 ANSWER 14 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 1999:974206 The Genuine Article (R) Number: 250YD. The safety and anti-platelet effects of CD39 after PTCA in pigs. Buergler J M (Reprint); Marcus A J; Maliszewski C R; Broekman M J; Schulz D G; Ali N M. BAYLOR COLL MED, VAMC, HOUSTON, TX 77030; NEW YORK VET AFFAIRS MED CTR, NEW YORK, NY; IMMUNEX RES & DEV CORP, SEATTLE, WA 98101. CIRCULATION (2 NOV 1999) Vol. 100, No. 18, Supp. [S], pp. 2486-2486. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621. ISSN: 0009-7322. Pub. country: USA. Language: English.
- L10 ANSWER 15 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 1999:974204 The Genuine Article (R) Number: 250YD. CD39 provides additive inhibition of platelet aggregation over aspirin and abciximab. Buergler J M (Reprint); Kaluza G L; Malisżewski C R; Ali N M. BAYLOR COLL MED, VAMC, HOUSTON, TX 77030; BAYLOR COLL MED, METHODIST HOSP, HOUSTON, TX 77030; IMMUNEX RES & DEV CORP, SEATTLE, WA 98101. CIRCULATION (2 NOV 1999) Vol. 100, No. 18, Supp. [S], pp. 2484-2484. Publisher: LIPPINCOTT WILLIAMS & WILKINS. 530 WALNUT ST, PHILADELPHIA, PA 19106-3621. ISSN: 0009-7322. Pub. country: USA. Language: English.
- L10 ANSWER 16 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 2000:257211 The Genuine Article (R) Number: 290FP. CD39/ECTO-adpase blocks and reverses human platelet reactivity: Significance for thrombosis . Marcus A J (Reprint); Drosopoulos J H P; Broekman M J; Gayle R B; Islam N; Buergler J; Ali M; Maliszewski C R. VET AFFAIRS MED CTR, NEW YORK, NY; CORNELL UNIV, WEILL MED COLL, NEW YORK, NY; BAYLOR COLL MED, HOUSTON, TX 77030; IMMUNEX CORP, SEATTLE, WA. THROMBOSIS AND HAEMOSTASIS (AUG 1999) Supp. [S], pp. 2161-2161. Publisher: F K SCHATTAUER VERLAG GMBH. P O BOX 10 45 43, LENZHALDE 3, D-70040 STUTTGART, GERMANY. ISSN: 0340-6245. Pub. country: USA. Language: English.

- L10 ANSWER 17 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 1999:973441 The Genuine Article (R) Number: 250YD. Cerebroprotective role of CD39 (endothelial EctoADPase) in murine stroke. McTaggart R A (Reprint); Broekman J; Peschon J; Stocking K; Choudhri T F; Kim L J; Connolly E S; Drosopoulos J H F; Maliszewski C R; Marcus A J; Pinsky D J. COLUMBIA UNIV, NEW YORK, NY; IMMUNEX RES & DEV CORP, SEATTLE, WA 98101; CORNELL UNIV, NEW YORK, NY. CIRCULATION (2 NOV 1999) Vol. 100, No. 18, Supp. [S], pp. 1720-1720. Publisher: LIPPINCOTT WILLIAMS & WILKINS . 530 WALNUT ST, PHILADELPHIA, PA 19106-3621. ISSN: 0009-7322. Pub. country: USA. Language: English.
- L10 ANSWER 18 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 2000:52763 The Genuine Article (R) Number: 257PH. Blockade and reversal of human platelet reactivity by CD39/ecto-ADPase. Potential for antithrombotic therapeusis. Marcus A J (Reprint); Broekman M J; Drosopoulos J H; Gayle R B; McTaggart R A; Pinsky D J; Islam N; Buergler J M; Ali M N; Maliszewski C R. VA NY HARBOR HLTH CARE, MED HEM ONC, NEW YORK, NY; CORNELL UNIV, WEILL MED COLL, NEW YORK, NY 10021; IMMUNEX RES & DEV CORP, SEATTLE, WA 98101; COLUMBIA UNIV, NEW YORK, NY; BAYLOR VA, CARDIOL, HOUSTON, TX. BLOOD (15 NOV 1999) Vol. 94, No. 10, Part 1, Supp. [1], pp. 1638-1638. Publisher: AMER SOC HEMATOLOGY. 1200 19TH ST, NW, STE 300, WASHINGTON, DC 20036-2422. ISSN: 0006-4971. Pub. country: USA. Language: English.
- L10 ANSWER 19 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 2000:45209 Document No.: PREV200000045209. Blockade and reversal of human platelet reactivity by CD39/ecto-ADPase. Potential for antithrombotic therapeusis. Marcus, A. J. [Reprint author]; Broekman, M. J. [Reprint author]; Drosopoulos, J. H. [Reprint author]; Gayle, R. B., III; McTaggart, R. A.; Pinsky, D. J.; Islam, N. [Reprint author]; Buergler, J. M.; Ali, M. N.; Maliszewski, C. R. Medicine Hem/Onc, VA NY Harbor Health Care, New York, NY, USA. Blood, (Nov. 15, 1999) Vol. 94, No. 10 SUPPL. 1 PART 1, pp. 368a. print. Meeting Info.: Forty-first Annual Meeting of the American Society of Hematology. New Orleans, Louisiana, USA. December 3-7, 1999. The American Society of Hematology.
- L10 ANSWER 20 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 2000:135870 Document No.: PREV200000135870. CD39/ecto-ADPase blocks and reverses human platelet reactivity. Significance for thrombosis. Marcus, Aaron J. [Reprint author]; Drosopoulos, Joan H. F.; Broekman, M. Johan; Gayle, Richard B., III; Islam, Naziba; Buergler, J.; Ali, M. N.; Maliszewski, Charles R.. VA New York Harbor Health Care System, 423 East 23rd Street, New York, NY, 10010, USA. Prostaglandins and Other Lipid Mediators, (Dec., 1999) Vol. 59, No. 1-6, pp. 73. print. Meeting Info.: 6th International Conference on Eicosanoids and other Bioactive Lipids in Cancer, Inflammation, and Related Diseases. Boston, Massachusetts, USA. September 12-15, 1999.

 ISSN: 1098-8823. Language: English.
- L10 ANSWER 21 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 1999:798910 The Genuine Article (R) Number: 238PX. CD39 provides additive inhibition of platelet aggregation over aspirin and abciximab. Buergler J M (Reprint); Maliszewski C; Kaluza G; Kleiman N; Cozart J; Ali M N. IMMUNEX CORP, SEATTLE, WA; VET AFFAIRS MED CTR, HOUSTON, TX 77030; BAYLOR COLL MED, HOUSTON, TX 77030. AMERICAN JOURNAL OF CARDIOLOGY (22 SEP 1999) Vol. 84, No. 6A, Supp. [S], pp. P67-P68. Publisher: EXCERPTA MEDICA INC. 245 WEST 17TH STREET, NEW YORK, NY 10011. ISSN: 0002-9149. Pub. country: USA. Language: English.
- L10 ANSWER 22 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN 2000:37019 Document No.: PREV200000037019. Cerebroprotective role of

CD39 (endothelial ectoADPase) in murine stroke. McTaggart, Ryan A. [Reprint author]; Broekman, M. Johan; Peschon, Jacques; Stocking, Kim; Choudhri, Tanvir F.; Kim, Louis J.; Connolly, E. Sander, Jr.; Drosopoulos, Joan H. F.; Maliszewski, Charles R.; Marcus, Aaron J.; Pinsky, David J. Columbia Univ, New York, NY, USA. Circulation, (Nov. 2, 1999) Vol. 100, No. 18 SUPPL., pp. I.328. print. Meeting Info.: 72nd Scientific Sessions of the American Heart Association. Atlanta, Georgia, USA. November 7-10, 1999. CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.

- ANSWER 23 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on L10 STN
- 2000:22278 Document No.: PREV200000022278. The safety and anti-platelet effects of CD39 after PTCA in pigs. Buergler, John M. [Reprint author]; Marcus, Aaron J.; Maliszewski, Charlie R.; Broekman, M. Johan; Schulz, Daryl G.; Ali, Nadir M. Baylor Coll of Medicine, Houston, TX, USA. Circulation, (Nov. 2, 1999) Vol. 100, No. 18 SUPPL., pp. I.472. print.

Meeting Info.: 72nd Scientific Sessions of the American Heart Association. Atlanta, Georgia, USA. November 7-10, 1999.

CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.

- L10 ANSWER 24 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on
- 2000:22276 Document No.: PREV200000022276. CD39 provides additive inhibition of platelet aggregation over aspirin and abciximab. Buergler, John M. [Reprint author]; Kaluza, Grzegorz L.; Maliszewski, Charles R.; Ali, Nadir M.. Baylor Coll of Medicine, Houston, TX, USA. Circulation, (Nov. 2, 1999) Vol. 100, No. 18 SUPPL., pp. I.472. print. Meeting Info.: 72nd Scientific Sessions of the American Heart Association. Atlanta, Georgia, USA. November 7-10, 1999. CODEN: CIRCAZ. ISSN: 0009-7322. Language: English.
- DUPLICATE 5 MEDLINE on STN L10 ANSWER 25 OF 35 Inhibition of platelet function by PubMed ID: 9576748. 1998244993. recombinant soluble ecto-ADPase/CD39. Gayle R B 3rd; Maliszewski C R; Gimpel S D; Schoenborn M A; Caspary R G; Richards C; Brasel K; Price V; Drosopoulos J H; Islam N; Alyonycheva T N; Broekman M J; Marcus A J. (Immunex Corporation, Seattle, Washington 98101, USA.. gayler@immunex.com) . Journal of clinical investigation, (1998 May 1) 101 (9) 1851-9. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
- Excessive platelet accumulation and recruitment, leading to vessel AB occlusion at sites of vascular injury, present major therapeutic challenges in cardiovascular medicine. Endothelial cell CD39, an ecto-enzyme with ADPase and ATPase activities, rapidly metabolizes ATP and ADP released from activated platelets, thereby abolishing recruitment. Therefore, a soluble form of CD39, retaining nucleotidase activities, would constitute a novel antithrombotic agent. We designed a recombinant, soluble form of human CD39, and isolated it from conditioned media from transiently transfected COS-1 cells and from stably transfected Chinese hamster ovary (CHO) cells. Conditioned medium from CHO cells grown under serum-free conditions was subjected to anti-CD39 immunoaffinity column chromatography, yielding a single approximately 66-kD protein with ATPase and ADPase activities. Purified soluble CD39 blocked ADP-induced platelet aggregation in vitro, and inhibited collagen-induced platelet reactivity. Kinetic analyses indicated that, while soluble CD39 had a Km for ADP of 5.9 microM and for ATP of 2.1 microM, the specificity constant kcat/Km was the same for both substrates. Intravenously administered soluble CD39 remained active in mice for an extended period of time, with an elimination phase half-life of almost 2 d. The data indicate that soluble CD39 is a potential therapeutic agent for inhibition of platelet-mediated thrombotic diatheses.
- L10 ANSWER 26 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- The Genuine Article (R) Number: 141AW. Characterization of the 1999:1265 structure and function of CD39. Gayle R B (Reprint);

- Gimpel S D; Maliszewski C R; Schoenborn M A; Caspary R G; DuBose R F; Ketchem R R; Johnson R S; Wallace A R; Drosopoulos J H F; Islam N; Broekman M J; Marcus A J. CORNELL UNIV, COLL MED, NEW YORK, NY; VET AFFAIRS MED CTR, NEW YORK, NY; IMMUNEX CORP, SEATTLE, WA. BLOOD (15 NOV 1998) Vol. 92, No. 10, Part 1, Supp. [1], pp. 696-696. Publisher: W B SAUNDERS CO. INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399. ISSN: 0006-4971. Pub. country: USA. Language: English.
- DUPLICATE 6 MEDLINE on STN L10 ANSWER 27 OF 35 PubMed ID: 9730622. Gene structure and chromosome location of 1998399871. mouse Cd39 coding for an ecto-apyrase. Schoenborn M A; Jenkins N A; Copeland N G; Gilbert D J; Gayle R B 3rd; Maliszewski C R. (Immunex Corporation, Seattle, WA, USA.. schoenborn@immunex.com) . Cytogenetics and cell genetics, (1998) 81 (3-4) 287-9. Journal code: 0367735. ISSN: 0301-0171. Pub. country: Switzerland. Language: English.
- L10 ANSWER 28 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on DUPLICATE 7 STN
- 1998:384932 Document No.: PREV199800384932. Endothelial cell CD39 /ecto-ADPASE, a novel platelet inhibitor. Marcus, A. J. [Reprint author]; Broekman, M. J.; Drosopoulos, J. H. F.; Islan, N.; Schoenborn, M. A.; Gimpel, S. D.; Gayle, R. B.; Maliszewski, C. R. . Dep. Veterans Affairs, Cornell Med. Coll., New York, NY, USA. Journal of Investigative Medicine, (March, 1998) Vol. 46, No. 3, pp. 232A. print. Meeting Info.: Annual Meeting of the Association of American Physicians, American Society for Clinical Investigation, American Federation for Medical Research 1998 Biomedicine: Medical Research from Bench to Bedside. Washington, D.C., USA. May 1-3, 1998. American Federation for Medical Research; American Society for Clinical Investigation; Association of American Physicians.
 - ISSN: 1081-5589. Language: English.
- L10 ANSWER 29 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 1999:96018 Document No.: PREV199900096018. Characterization of the structure and function of CD39. Gayle, R. B., III [Reprint author]; Gimpel, S. D. [Reprint author]; Maliszewski, C. R. [Reprint author]; Schoenborn, M. A. [Reprint author]; Caspary, R. G. [Reprint author]; Dubose, R. F. [Reprint author]; Ketchem, R. R. [Reprint author]; Johnson, R. S. [Reprint author]; Wallace, A. R. [Reprint author]; Drosopoulos, J. H. F. [Reprint author]; Islam, N. [Reprint author]; Broekman, M. J. [Reprint author]; Marcus, A. J.. Immunex Corp., Seattle, WA, USA. Blood, (Nov. 15, 1998) Vol. 92, No. 10 SUPPL. 1 PART 1-2, pp. 172A-173A. print. Meeting Info.: 40th Annual Meeting of the American Society of Hematology. Miami Beach, Florida, USA. December 4-8, 1998. The American Society of

Heamatology. CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

- DUPLICATE 8 L10 ANSWER 30 OF 35 MEDLINE on STN The endothelial cell ecto-ADPase responsible PubMed ID: 9077545. 97232270. for inhibition of platelet function is CD39. Marcus A J; Broekman M J; Drosopoulos J H; Islam N; Alyonycheva T N; Safier L B; Hajjar K A; Posnett D N; Schoenborn M A; Schooley K A; Gayle R B ; Maliszewski C R. (Department of Medicine, Veterans Affairs Medical Center, New York 10010-5050, USA.. ajmarcus@mail.med.cornell.edu) . Journal of clinical investigation, (1997 Mar 15) 99 (6) 1351-60. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Lanquage: English.
- We previously demonstrated that when platelets are in motion and in AB proximity to endothelial cells, they become unresponsive to agonists (Marcus, A.J., L.B. Safier, K.A. Hajjar, H.L. Ullman, N. Islam, M.J. Broekman, and A.M. Eiroa. 1991. J. Clin. Invest. 88:1690-1696). inhibition is due to an ecto-ADPase on the surface of endothelial cells which metabolizes ADP released from activated platelets, resulting in blockade of the aggregation response. Human umbilical vein endothelial cells (HUVEC) ADPase was biochemically classified as an E-type ATP-diphosphohydrolase. The endothelial ecto-ADPase is herein identified

as CD39, a molecule originally characterized as a lymphoid surface antigen. All HUVEC ecto-ADPase activity was immunoprecipitated by monoclonal antibodies to CD39. Surface localization of HUVEC CD39 was established by confocal microscopy and flow cytometric Transfection of COS cells with human CD39 resulted in both ecto-ADPase activity as well as surface expression of CD39. PCR analyses of cDNA obtained from HUVEC mRNA and recombinant human CD39 revealed products of the same size, and of identical sequence. Northern blot analyses demonstrated that HUVEC express the same sized transcripts for CD39 as MP-1 cells (from which CD39 was originally cloned). We established the role of CD39 as a prime endothelial thromboregulator by demonstrating that CD39-transfected COS cells acquired the ability to inhibit ADP-induced aggregation in platelet-rich plasma. The identification of HUVEC ADPase/CD39 as a constitutively expressed potent inhibitor of platelet reactivity offers new prospects for antithrombotic therapeusis.

- L10 ANSWER 31 OF 35 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
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- L10 ANSWER 32 OF 35 CAPLUS COPYRIGHT 2005 ACS on STN
- 1997:626537 Document No. 127:290941 Control of platelet reactivity by an ecto-ADPase on human endothelial cells. Marcus, A. J.; Broekman, M. J.; Drosopoulos, J. H. F.; Islam, N.; Alyonycheva, T.; Safier, L. B.; Hajjar, K. A.; Posnett, D. N.; Schoenborn, M. A.; Schooley, K.; Maliszewski, C. R. (Div. Hematology/Oncology, Dep. Med., Dep. Veterans Affairs Med. Cent., Division Hematology/Oncology, Dep. Med., Ped., Pathol., Cornell Univ. Med. Coll., New York, NY, USA). Ecto-ATPases: Recent Progress on Structure and Function, [Proceedings of the International Workshop on Ecto-ATPases]. 1st, Mar del Plata, Argentina, Aug. 26-30, 1996, Meeting Date 1996, 167-170. Editor(s): Plesner, Liselotte; Kirley, Terence L.; Knowles, Aileen F. Plenum: New York, N. Y. (English) 1997. CODEN: 65DBAR.
- AB A review, with 15 refs., on thromboregulation, biochem. properties of human umbilical vein endothelial cell (HUVEC) ecto-ADPase, and HUVEC ecto-ADPase/CD39.
- L10 ANSWER 33 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 97:632134 The Genuine Article (R) Number: XE898. Inhibition of platelet reactivity by human endothelial cell ecto-ADPase/CD39. Marcus A J (Reprint); Broekman M J; Drosopoulos J H F; Islam N; Alyonycheva T; Safier L B; Hajjar K A; Posnett D N; Schoenborn M A; Schooley K; Maliszewski C R. IMMUNEX CORP, SEATTLE, WA; CORNELL UNIV MED COLL, DEPT PEDIAT, NEW YORK, NY; CORNELL UNIV MED COLL, DEPT PATHOL, NEW YORK, NY; CORNELL UNIV MED COLL, DEPT MED, DIV HEMATOL ONCOL, NEW YORK, NY. THROMBOSIS AND HAEMOSTASIS (JUN 1997) Supp. [S], pp. SC15-SC15. Publisher: F K SCHATTAUER VERLAG GMBH. P O BOX 10 45 45, LENZHALDE 3, D-70040 STUTTGART, GERMANY. ISSN: 0340-6245. Pub. country: USA. Language: English.
- L10 ANSWER 34 OF 35 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
- 96:888670 The Genuine Article (R) Number: VT983. CD39 is the endothelial cell ecto-ADPpase responsible for inhibition of platelet function. Marcus A J (Reprint); Broekman M J; Drosopoulos J H F; Islam N; Alyonycheva T; Safier L B; Hajjar K A; Posnett D N; Schoenborn M A;

Schooley K; Maliszewski C R. CORNELL UNIV MED COLL, DEPT VET AFFAIRS MED CTR, DEPT MED, DIV HEMATOL ONCOL, SEATTLE, WA; CORNELL UNIV MED COLL, DEPT PEDIAT, SEATTLE, WA; CORNELL UNIV MED COLL, DEPT PATHOL, SEATTLE, WA; IMMUNEX CORP, SEATTLE, WA. BLOOD (15 NOV 1996) Vol. 88, No. 10, Part 1, Supp. [1], pp. 1850-1850. Publisher: W B SAUNDERS CO. INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399. ISSN: 0006-4971. Pub. country: USA. Language: English.

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95015846. PubMed ID: 7930580. The CD39 lymphoid cell activation
antigen. Molecular cloning and structural characterization.
Maliszewski C R; Delespesse G J; Schoenborn M A; Armitage R J;
Fanslow W C; Nakajima T; Baker E; Sutherland G R; Poindexter K; Birks C;
+. (Immunex Research and Development Corporation, Seattle, WA 98101.)
Journal of immunology (Baltimore, Md.: 1950), (1994 Oct 15) 153 (8)
3574-83. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United
States. Language: English.

CD39, a 70- to 100-kDa molecule expressed primarily on activated AB lymphoid cells, was previously shown to mediate B cell homotypic adhesion when ligated with a subset of anti-CD39 mAbs. In the present study, we describe the cloning and molecular characterization of human and murine CD39. The nucleotide sequence of human CD39 includes an open reading frame encoding a putative 510 amino acid protein with six potential N-linked glycosylation sites, 11 Cys residues, and two potential transmembrane regions. Murine CD39 shares 75% amino acid sequence identity with human CD39 but fails to cross-react with anti-human CD39 mAbs. Although there were no significant similarities with other mammalian genes, considerable homology was found between CD39 and a guanosine diphosphatase from yeast. A series of mouse-human hybrid molecules was constructed to determine the general topology of CD39 and the location of a biologically functional epitope. These findings and supporting evidence from an anti-CD39 mAb-selected phage peptide display library indicate a likely model wherein a short intracellular N-terminus is followed by a large extracellular loop containing the epitope recognized by stimulatory anti-CD39 mAbs, and a short intracellular C terminus. The results demonstrate that CD39 is a novel cell surface glycoprotein with unusual structural characteristics.

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